Supplemental Data

HNF1α suppresses steatosis associated liver cancer by inhibiting PPARγ transcription

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Supplemental methods

Gene expression analysis of human HCC samples. Gene expression datasets GSE14520 and GSE36376 were obtained from the GEO website (http://www.ncbi.nlm.nih.gov/geo/). Affymetrix raw data (CEL files) from GSE14520 dataset were normalized in batch using the RMA method implemented in the R package affy (using default options), yielding log2 intensity values. Expression values of PPARγ were based on the mean of the three following probe sets: 208510_s_at, 202934_at and 201251_at. Illumina normalized log2 intensities expression data from the GSE36376 dataset were used. Expression measures of PPARγ were based on the mean of the four following probes: ILMN_1687612, ILMN_1800225, ILMN_2364384, ILMN_1679901.

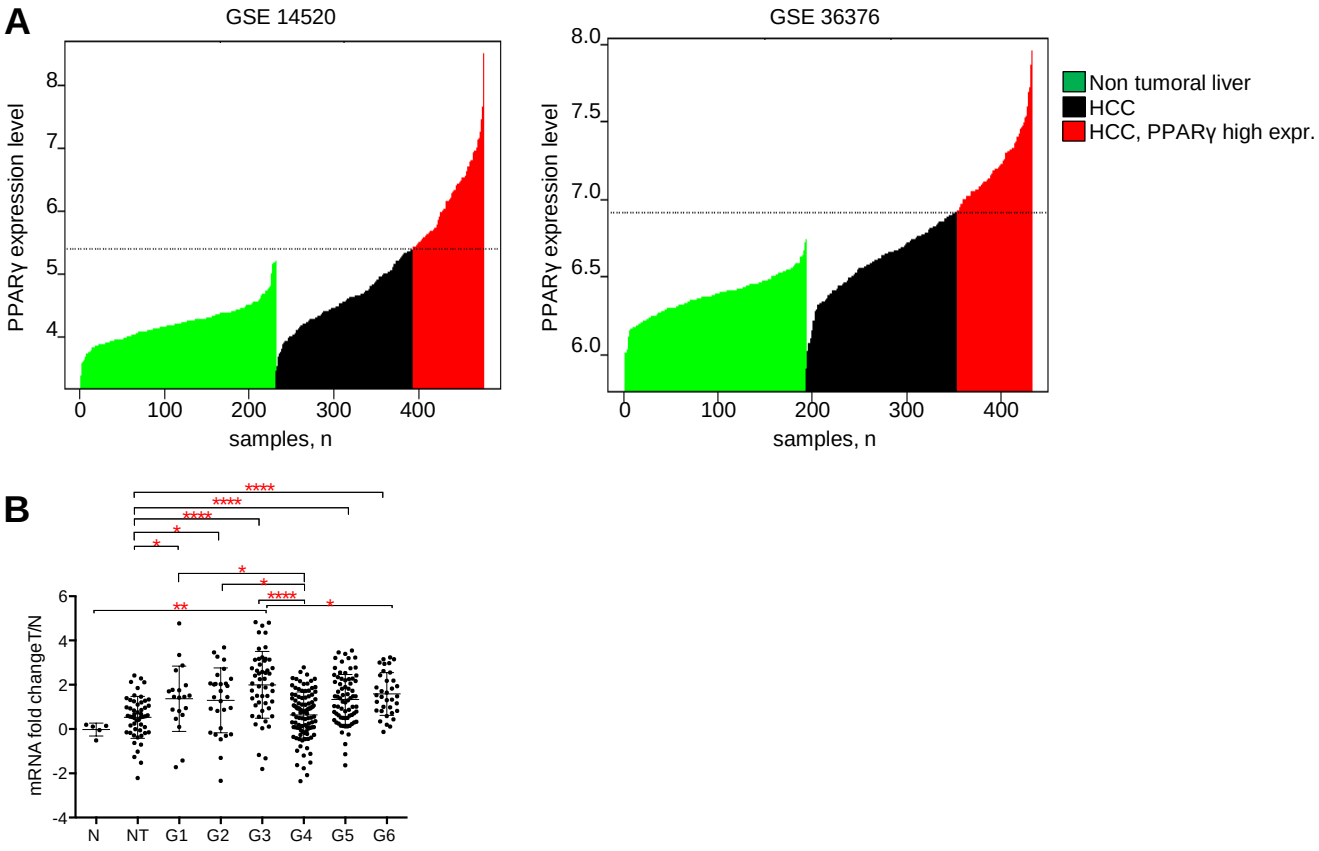
Microscopy. For fluorescent microscopy analyses, HUH7 cells were transfected with pcDNA5-Myc-HNF1A (Addgene #31104) and pcDNA5-Myc-HNF1A-S247D using Lipofectamine 2000 (Invitrogen). 24 hours after cells were plated on coverslips (Millipore) and treated as follows: for starvation, cells were washed twice with PBS before changing medium with DMEM containing only antibiotics for 16 hours; for stimulation, starvation media was supplemented with 10% FBS for 30 minutes; for inhibitor treatments, Torin was added to a final concentration of 100nM for 30 minutes before stimulation with 10% FBS for 30 minutes. HUH7 cells were fixed with 4% paraformaldehyde in PBS for 20 minutes and permeabilized with 0.01% TritonX100 in PBS for 10 min, followed by blocking in 3% BSA in PBS supplemented with 0.15% donkey serum. Slides were then treated with anti-MYC antibody overnight at +4C. Secondary antibody used for this assay was anti-mouse IgG Alexa Fluor 488 (Invitrogen). DAPI counterstaining was used to visualize nuclei. Images were acquired with an optical slice of 0.8 µm using a 40×/0.75 oil immersion objective using an Apotome 2 CO2 microscope (Zeiss) and analyzed using ZEN software (Zeiss). All samples for microscopy were viewed at room temperature.

Quantifications were performed by classifying at least 200 cells in groups of nuclear, cytoplasmic or nuclear/cytoplasmic signal for HNF1α. Calculations are the average of the results of three independent experiments.

Chromatin immunoprecipitation. HUH7 cells were plated in 70% confluence in Dulbecco's modified Eagle's medium supplemented with 10% FBS, 2 mM L-glutamine, 50 units/ml penicillin, and 50 µg/ml streptomycin 24 hour before collection. Crosslinking was performed on plates by adding formaldehyde to a final concentration of 1% for 10 minutes and cells were incubated at room temperature with shaking at 100rpm. Crosslinking was terminated by the addition of glycine to a final concentration of 125mM. After washing with ice-cold PBS, cells were collected and resuspended in LysB22 buffer (50 mM TRIS-HCl pH 8.0, 150 mM NaCl, 25 mM EDTA, 1% Triton, and 0.3% SDS with complete protease inhibitors (Roche) followed by sonication using a Bioruptor Pico (Diagenode). Endogenous HNF1α was immunoprecipitated with anti-HNF1α antibody (63). The anti-rabit IgG antibody was used as a control. Immunocomplexes were recovered with Dynabeads protein A (Life Technologies) followed by five successive washes (I:1% Triton, 0.1% Sodium Deoxycholate, 150 mM NaCl, 10 mM TRIS-HCl pH8; II: 1% NP-40, 1% Sodium Deoxycholate, 150 mM KCl, 10 mM TRIS-HCl pH8; III: 0.5% Triton, 0.1% Sodium Deoxycholate, 500 mM NaCl, 10 mM TRIS-HCl pH8; IV: 0.5% NP-40, 0.5% Sodium Deoxycholate, 250 mM LiCl, 20 mM TRIS-HCl pH8, 1 mM EDTA; V: 0.1% NP-40, 150 mM NaCl, 20 mM TRIS-HCl pH8, 1 mM EDTA) and twice with TE buffer (10 mM Tris-HCl pH8, 1 mM EDTA pH8). DNA–protein complexes were eluted by Elution Buffer (1% SDS, 0.1M NaHCO3, 100mM NaCl) and cross-linking was reversed by heating the samples at 65 °C for 16 h in the presence of 20µg/ml RNase A, and then treated with 100µg/ml of proteinase K at 55°C for 2 hours. DNA was purified using the Qiaquick PCR purification kit (Qiagen). Real-time quantitative PCR was performed on MX3005P

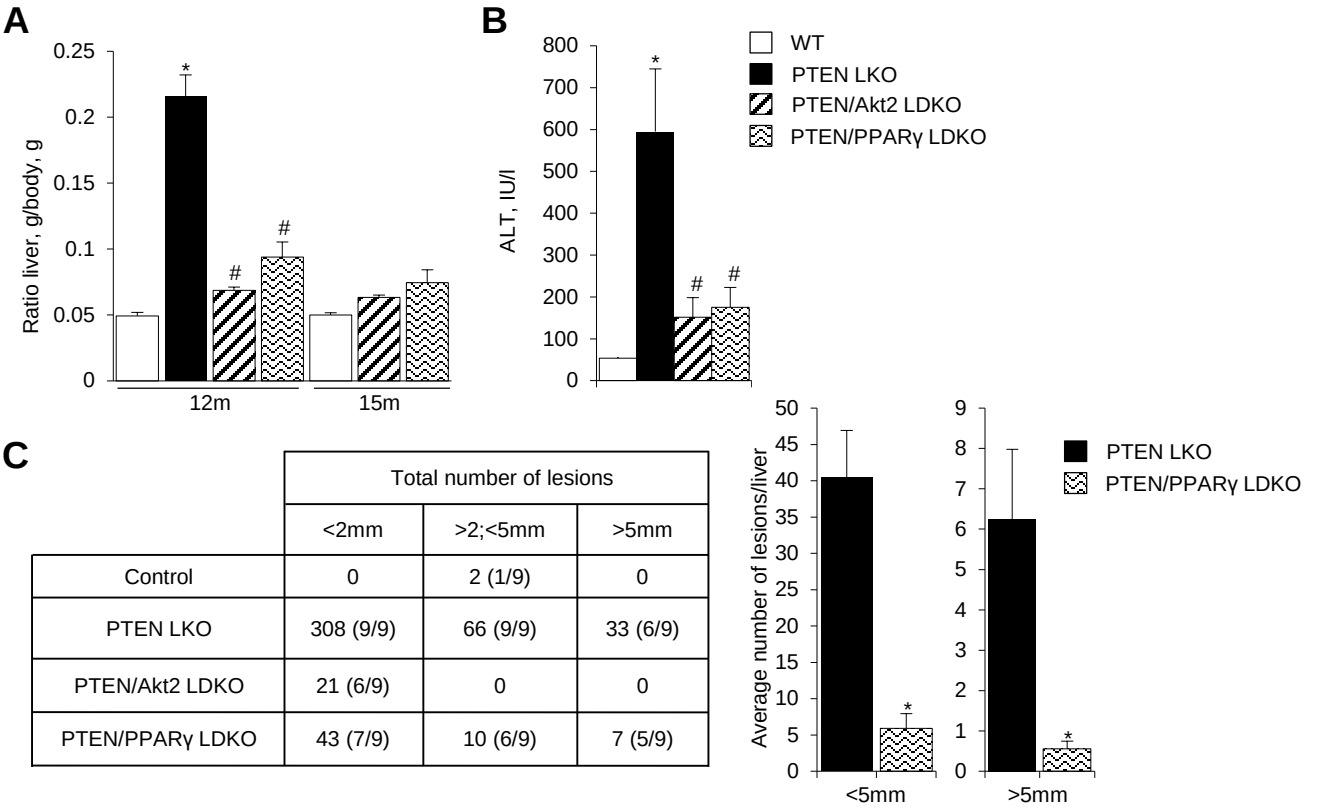
instrument (Agilent) using a iTaq Universal SYBR Green Supermix (Biorad).The relative amounts of the immunoprecipitated DNA were determined by means of the $2^{-\Delta\Delta CT}$ method, with input DNA values for each sample as control. The enrichment over IgG control of more than tenfold was considered as a cut-off. The location of putative HREs within the *PPARG* promoter region and their conservation in different species was determined using MatInspector software (Genomatix), the UCSC Genome Browser and Clustal Omega Multiple Sequence Alignment (EMBL-EBI). Primer pairs flanking putative HREs used for amplification were designed using PrimerBlast software. Calculations are the mean of fold difference of three independent experiments. The primer sequences are listed in **Supplemental Table 2**.

Supplemental figure 1

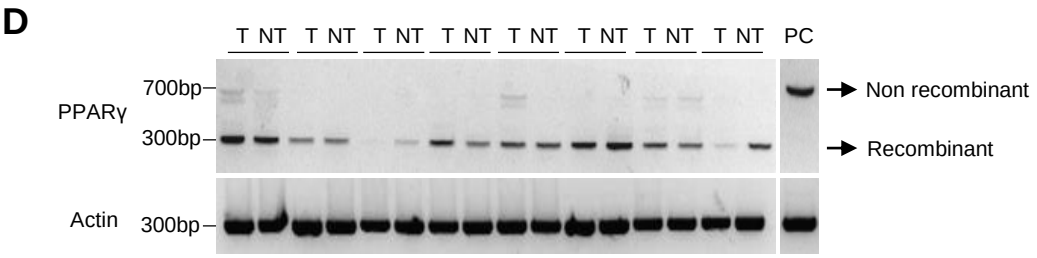


Supplemental figure 1. PPARγ is induced in a subset of human HCCs. (**A**) Analysis of *PPARG* transcript levels in microarray data sets of human HCCs from NCBI GEO DataSets. The dotted line represents the mean expression level. GSE 14520 number of samples: HCC (n=246) and Non-Tumoral liver tissue (n=231). GSE 36376 number of samples: HCC (n=240) and adjacent Non-Tumoral liver tissue (n=193). (**B**) Expression profiles of *PPARG* transcript (*Hs00234592_m1*) in control Normal liver (n=5), Non-Tumoral liver (n=52) and in HCC groups G1 (n=21), G2 (n=30), G3 (n=51), G4 (n=105), G5 (n=73), G6 (n=35) by qRT-PCR. Data are presented as the mean fold (log2) compared to the mean value in non-tumoral samples±SEM. Statistical analysis was performed with the Mann-Whitney test, *:p< 0.05; **:p< 0.01; ***:p< 0.001.

Supplemental figure 2

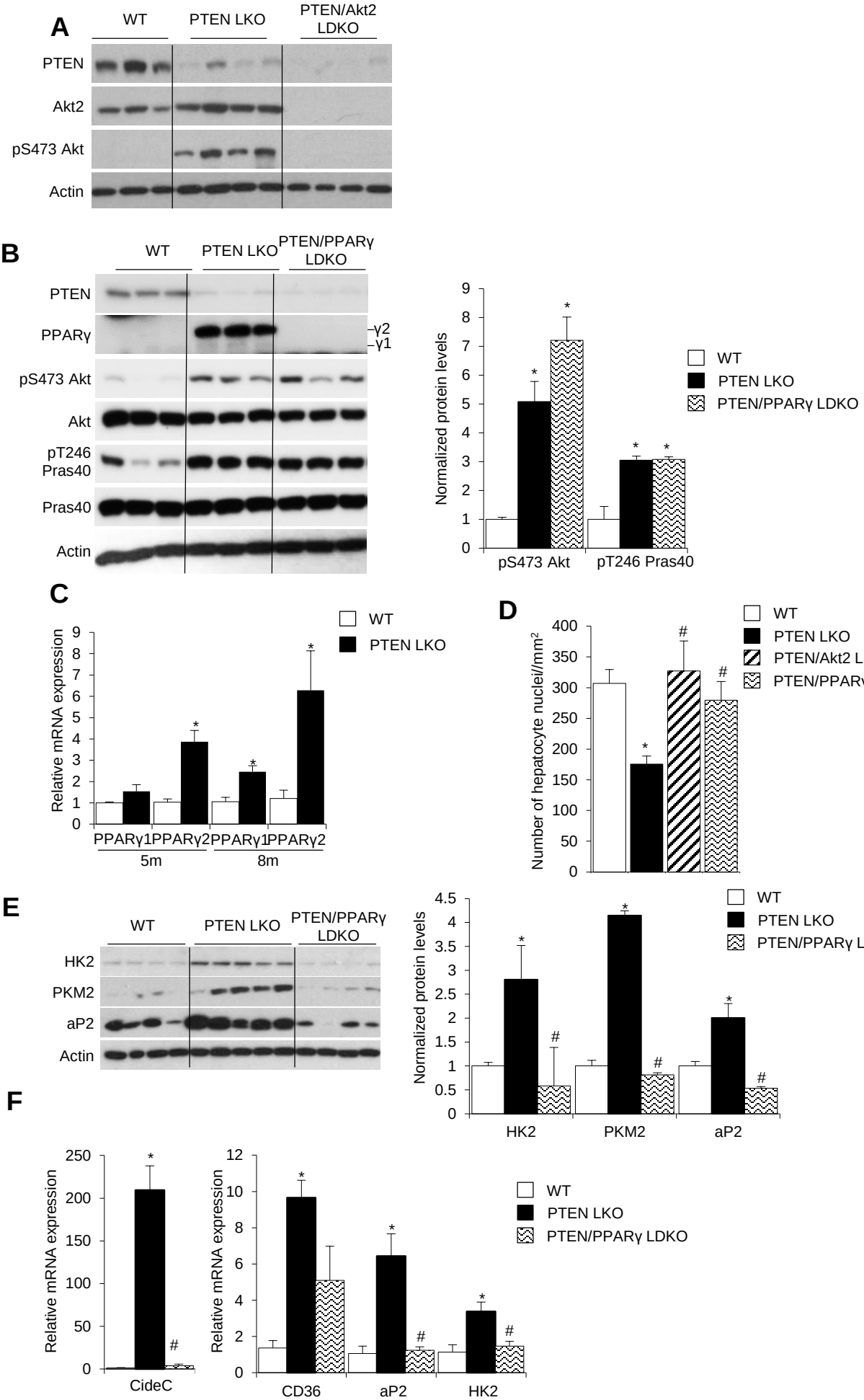


	Total number of lesions		
	<2mm	>2;<5mm	>5mm
Control	0	2 (1/9)	0
PTEN LKO	308 (9/9)	66 (9/9)	33 (6/9)
PTEN/Akt2 LDKO	21 (6/9)	0	0
PTEN/PPARγ LDKO	43 (7/9)	10 (6/9)	7 (5/9)



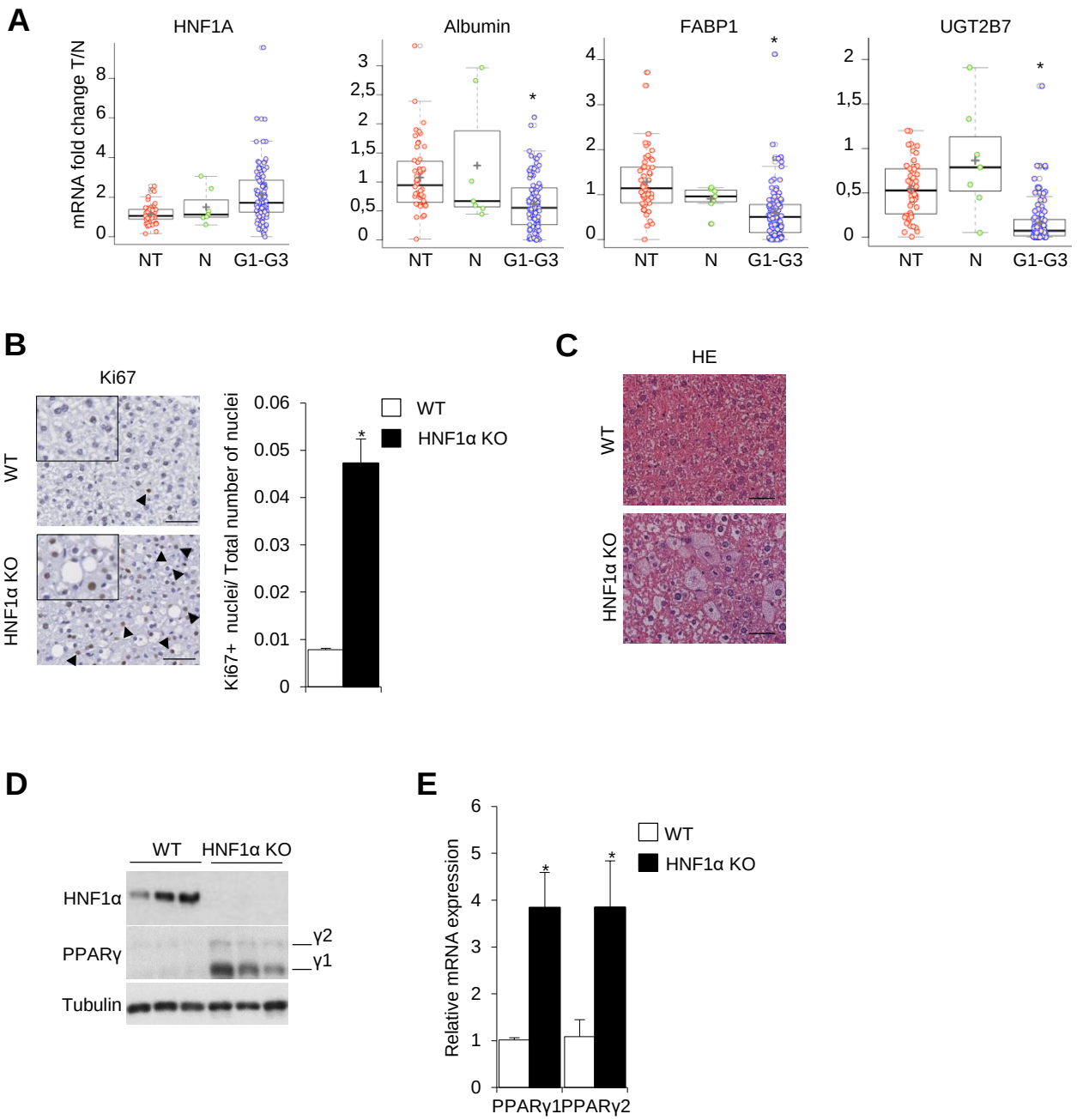
Supplemental figure 2. Deletion of *AKT2* and *PPARG* rescues hepatic phenotypes of *PTEN* mutants. (**A**) Relative liver weight in random fed male mice of indicated age and genotypes (n=9). (**B**) Plasmatic alanine transaminase levels in random fed 12 month old male mice. Data are means ±SEM, n=9. *:p<0.05 vs WT and #:p<0.05 vs *PTEN* LKO; 1-way ANOVA with Tukey's multiple comparisons test. (**C**) Table representing tumour incidence categorised in three groups by size in 12 month old male mice of indicated genotypes. Graph represents an average number of macroscopic lesions per liver in 12 month old *PTEN* LKO and *PTEN/PPARG* LDKO mice separated to two groups according to size (n=9). (**D**) RT-PCR of recombination in *PPARG* locus in tumoral (T) and non-tumoral (NT) samples from *PTEN/PPARG* LDKO mice. For the analyses, cDNA was synthesised using total RNA prepared from snap frozen tissue and recombination in *PPARG* locus was evaluated by PCR using specific primers. PCR products were resolved on agarose gel containing ethidium bromide. WT liver is used as positive control (PC). Actin is used as a control.

Supplemental figure 3



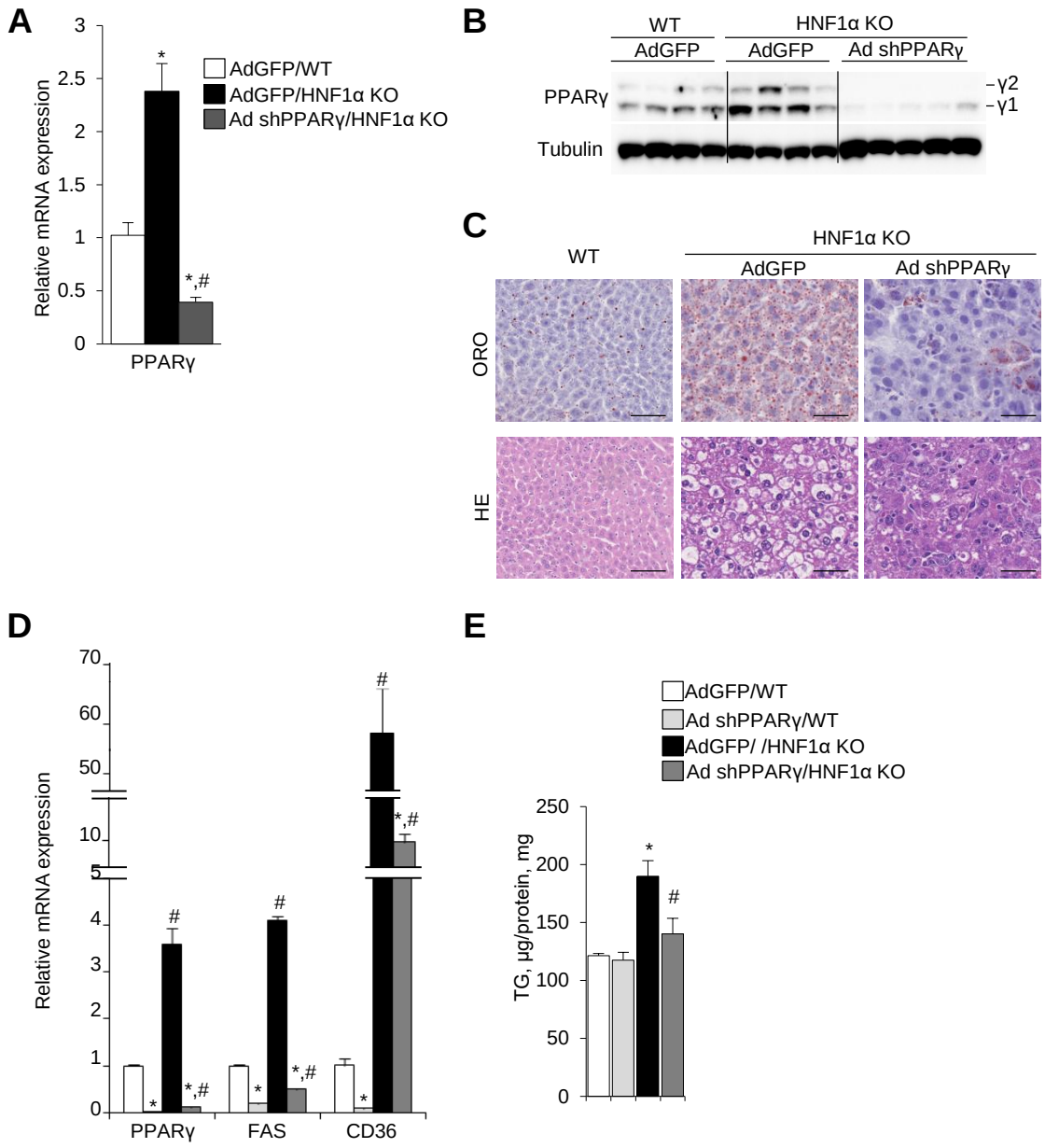
Supplemental figure 3. Deletion of *PPARG* rescues hepatic phenotypes of *PTEN* mutants. (**A** and **B**) Immunoblot analysis of total protein extracts in livers of 5 month old male mice of indicated genotypes. Densitometric analysis of Actin normalised signals is presented as a graph. Data are means ±SEM, n=4-7. *:p<0.05 vs WT, 1-way ANOVA with Tukey's multiple comparisons test. (**C**) Relative transcript levels of PPARγ1 and PPARγ2 isoforms in liver tissue of 5 and 8 month old random-fed male mice. Data are means ±SEM, n=4-7. *:p<0.05 vs WT, 2-tailed, unpaired Student's t test. (**D**) Hepatocyte size represented as nuclei density in a total area of 0.3 mm^2 of tissue in livers of 5 month old male mice of indicated genotypes. Data are means±SEM, n=3-10. *:p<0.05 vs WT and #:p<0.05 vs *PTEN* LKO; 2-tailed, unpaired Student's t test. (**E** and **F**) Immunoblot analysis of total protein extracts, densitometric analysis of Actin normalised signals is presented as a graph (**E**) and relative transcript levels of PPARγ target genes (**F**). Data are means±SEM, n=3-10. *:p<0.05 vs WT and #:p<0.05 vs *PTEN* LKO; 1-way ANOVA with Tukey's multiple comparisons test.

Supplemental figure 4



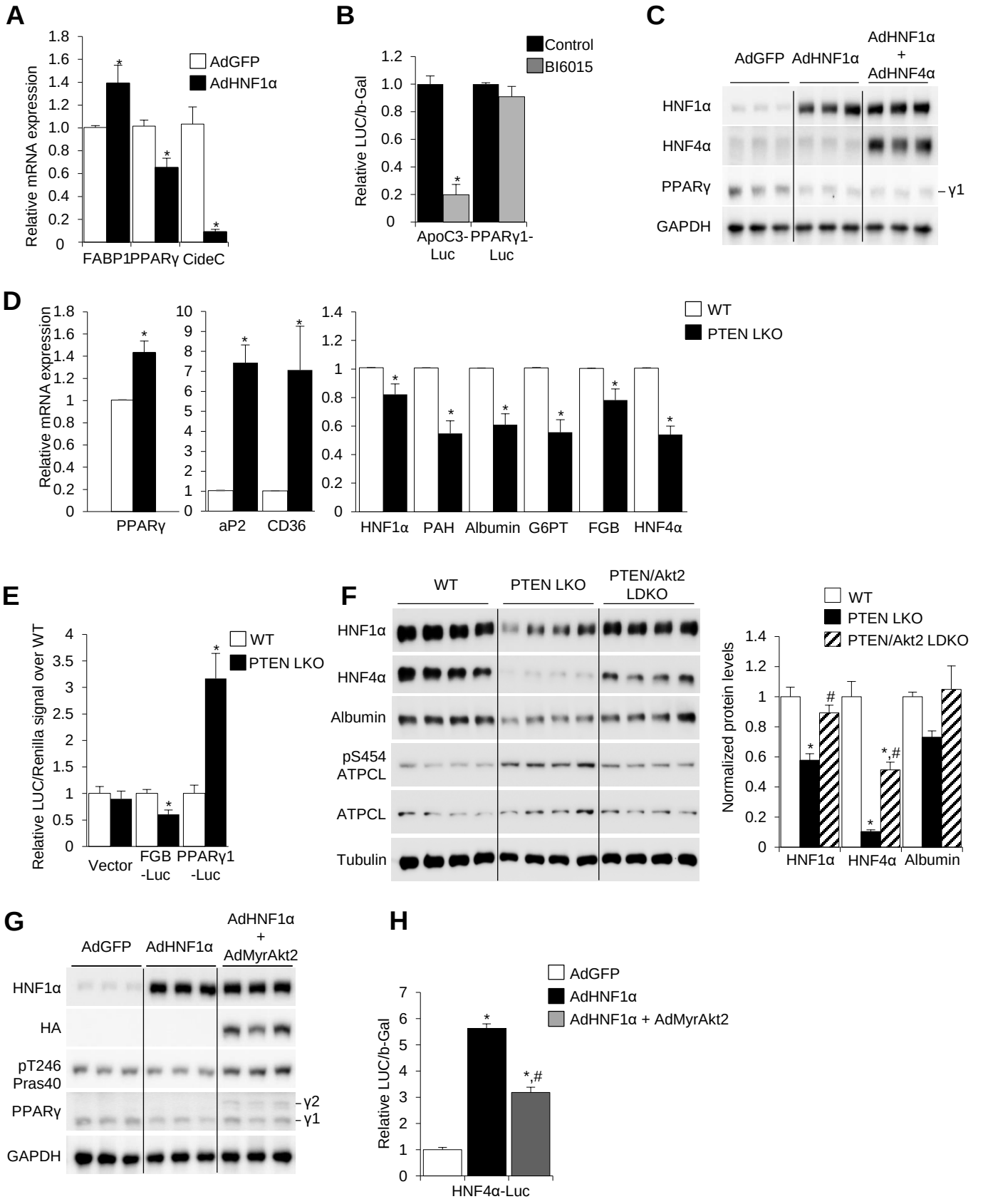
Supplemental figure 4. PPARγ is required for HNF1α driven steatosis. (**A**) Expression profiles of *HNF1A* transcript and transcript levels of HNF1α target genes in Non-Tumoral liver (n=52), control Normal liver (n=7), and in HCC groups G1-G3 (n=102) by qRT-PCR. Data are presented as a ribosomal 18S normalized mean fold (log2) compared to the mean value in non-tumoral samples±SEM. Statistical analysis was performed with the Mann-Whitney test, *:p<0.05 vs N. (**B**-**E**) Hepatocyte proliferation revealed by anti-Ki67 immunohistochemistry and analyzed as a ratio of Ki67$^+$ nuclei to total number of hepatocyte nuclei (n=3) (scale bar, 25 µm, the inset shows the magnified view of the Ki67$^+$ positive hepatocytes) (**B**), representative H&E-stained section, scale bar: 50 µm (**C**), immunoblot analysis of HNF1α and PPARγ protein levels (immunoblot with anti-Tubulin antibody served as a loading control) (**D**), relative transcript levels of PPARγ1 and PPARγ2 isoforms (**E**) in livers of 3 month old random-fed WT and *Hnf1a* mutant male mice. Data are means±SEM, n=3-5. *: p<0.05 vs WT; 2-tailed, unpaired Student's t test.

Supplemental figure 5



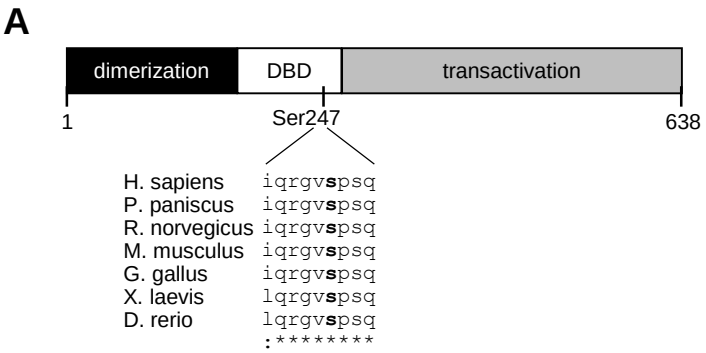
Supplemental figure 5. PPARγ is required for HNF1α driven steatosis. (**A**-**C**) Relative transcript levels of *PPARG* (**A**), immunoblot analysis of PPARγ levels (**B**) and representative ORO and H&E-stained sections (**C**) of livers of 10 week old random-fed female mice sacrificed 5 days post transduction with adenovirus expressing PPARγ shRNA or GFP as a control. Scale bar, 50 µm. Data are means±SEM, n=4-5. *: p<0.05 vs WT/AdGFP and #:p<0.05 vs *Hnf1a* KO/AdGFP; 1-way ANOVA with Tukey's multiple comparisons test. (**D** and **E**) Relative transcript levels of *PPARG* and its target genes (**D**) and triglyceride content (**E**) in primary hepatocytes isolated from 2 month old wild type and *Hnf1a* KO mice transduced with adenovirus expressing PPARγ shRNA or GFP. Data are means±SEM, n=3. *:p<0.05 vs WT, #: p<0.05 vs AdGFP; 1-way ANOVA with Tukey's multiple comparisons test.

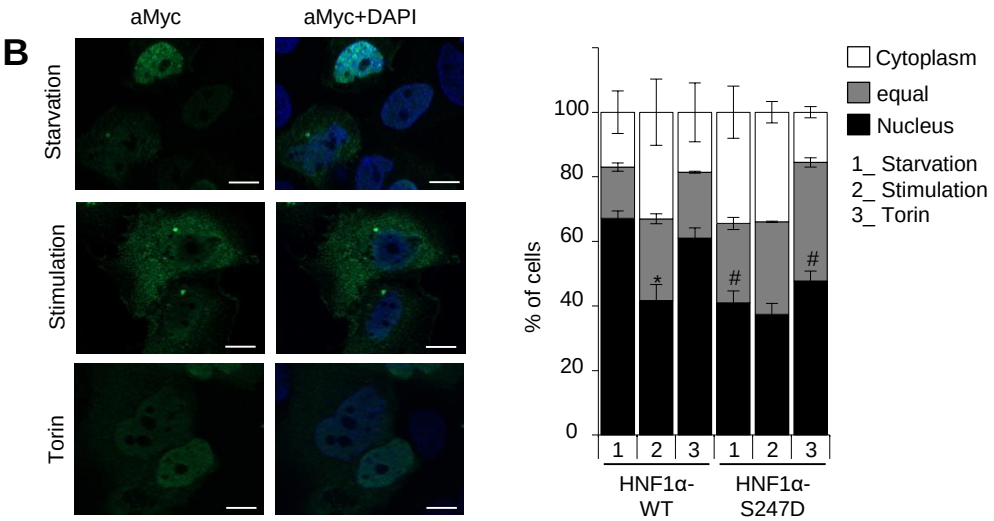
Supplemental figure 6

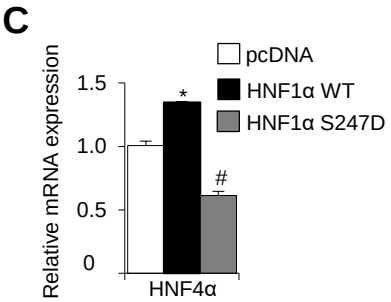


Supplemental figure 6. HNF1α is inhibited by Akt2. (**A**) Relative transcript levels of *PPARG*, *CideC* and *FABP1* in primary hepatocytes upon HNF1α overexpression analysed 24 hours posttransduction. Data are means±SEM, n=3. *: p<0.05 vs AdGFP; 2-tailed, unpaired Student's t test. (**B**) Luciferase reporter activity of *ApoC3* or *PPARG* promoter constructs normalized to β-Galactosidase activity in primary hepatocytes treated with 20uM BI6015 for 24 hours. Data are means ±SEM, n=3. *: p<0.05 versus control; 2-tailed, unpaired Student's t test. (**C**) Immunoblot analysis of total protein extracts from primary hepatocytes transduced with adenoviral vectors overexpressing GFP, HNF1α or HNF1α and HNF4α collected 36 hours post-infection. (**D**) Relative transcript levels of *PPARG*, *Hnf1a* and their target genes from primary hepatocytes isolated from 3 month old male mice. Data are means±SEM, n=4-5. *:p<0.05 vs WT, 2-tailed, unpaired Student's t test. (**E**) Luciferase reporter activity of FGB-LUC or *PPARG* promoter constructs normalized to Renilla signal in primary hepatocytes isolated from 3 month old male mice. Data are means ±SEM, n=3 independent hepatocyte cultures. Data are presented as fold difference over empty vector transfected wild-type hepatocytes. *: p<0.05 vs WT; 2-tailed, unpaired Student's t test. (**F**) Immunoblot analysis of total protein extracts from primary hepatocytes isolated from 3 month old male mice of indicated genotypes. Densitometric analysis of Actin normalized signals is presented as a graph. *: p<0.05 vs WT; #:p<0.05 vs *PTEN* LKO, 1-way ANOVA with Tukey's multiple comparisons test. (**G** and **H**) Immunoblot analysis of total protein extracts (**G**) and luciferase reporter activity of *HNF4A* promoter construct normalized to β-Galactosidase activity (**H**) in primary hepatocytes transduced with adenoviral vectors overexpressing GFP, HNF1α or a combination of HNF1α and Myr-Akt2 collected 36 hours post-infection. Data are means±SEM, n=3 independent experiments. *:p<0.05 vs AdGFP and #: p<0.05 vs AdHNF1α; 1-way ANOVA with Tukey's multiple comparisons test.

Supplemental figure 7

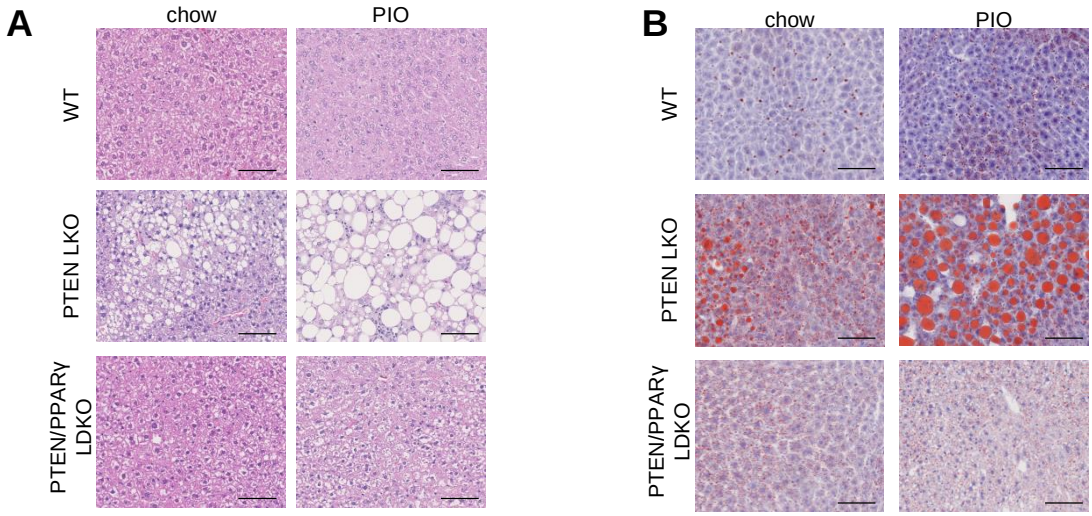


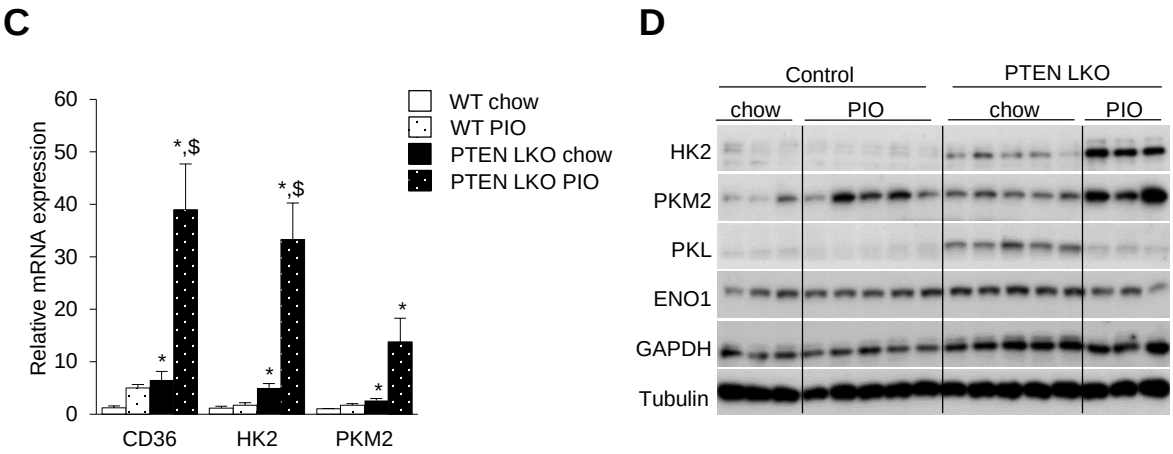




Supplemental figure 7. Phosphorylation of Ser247 in HNF1α is regulatory. (**A**) Schematic representation of domain structure of HNF1α protein (DBD-DNA binding domain). The evolutionary conservation of Ser247 and surrounding region is shown. (**B**) Representative images of indirect immunofluorescence analyses of transiently overexpressed Myc-tagged HNF1α-WT and HNF1α-S247D proteins in HUH7 cells. 24 hours post transfection, HUH7 cells were serum starved overnight followed by 30 min treatment with 100nM Torin before 30 min stimulation with 10% FBS. Cells were formalin fixed and stained with anti-Myc antibody. Secondary anti-mouse IgG Alexa Fluor 448 antibody were used for detection. Nuclei were counterstained with DAPI. Nuclear, cytoplasmic or equally distributed nuclear-cytoplasmic localization of overexpressed HNF1α-Myc protein was scored in 200-300 transfected cells. The quantification represents the fractions of cells showing specific HNF1α-Myc localization in each category scored. Data are means ±SEM, n=3. *: $p<0.05$ vs starvation, #: $p<0.05$ vs HNF1α-WT, 2-tailed, unpaired Student's t test. Scale bar, 12.5 µm. (**C**) Relative transcript level of HNF4α in HUH7 cells transiently overexpressing Myc-tagged HNF1α-WT or HNF1α-S247D proteins. Cells were collected 24 hours post transfection. Data are means ±SEM, n=3 independent experiments in triplicates. *: $p<0.05$ versus pcDNA and #: $p<0.05$ vs HNF1α WT; 2-tailed, unpaired Student's t test.

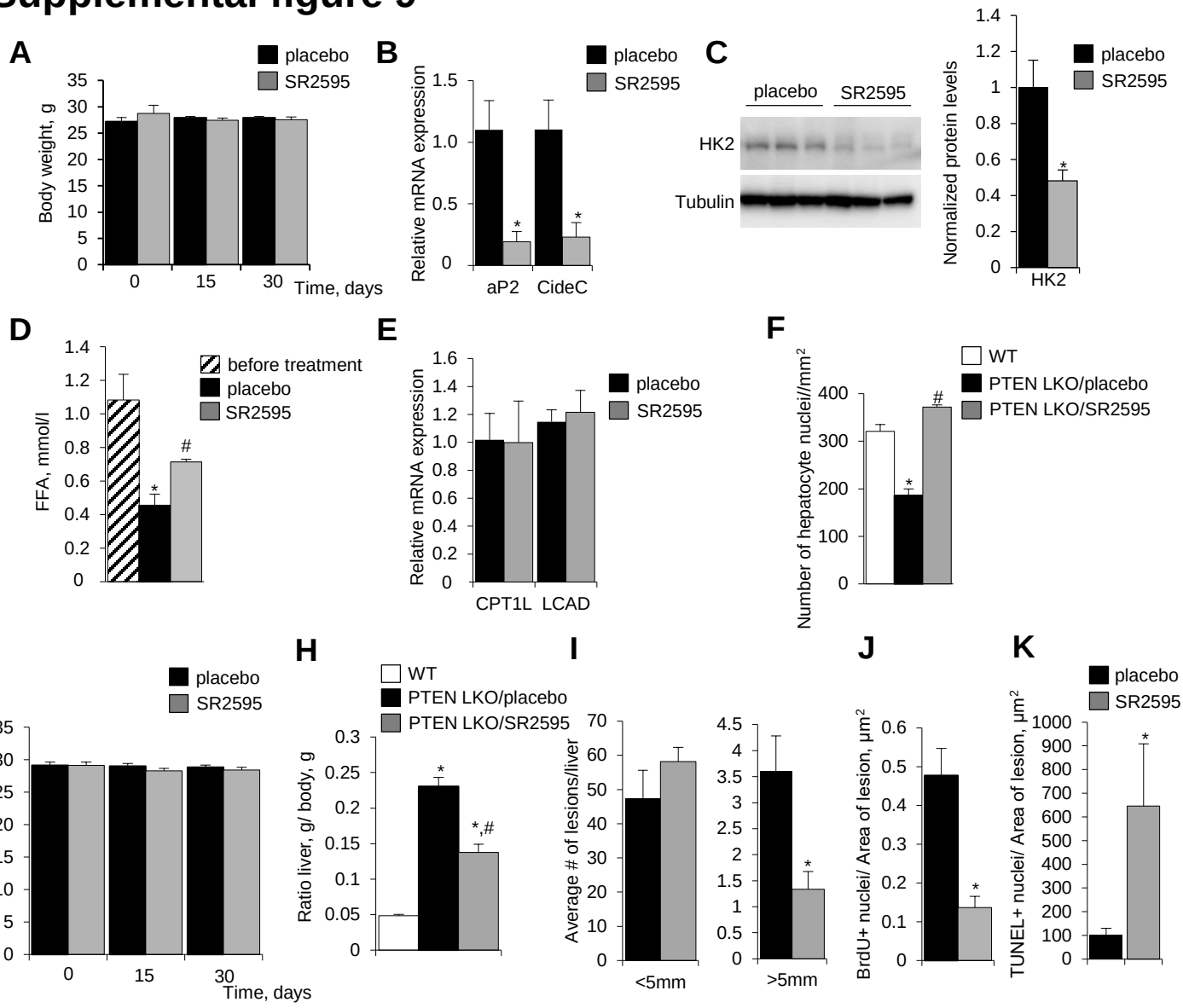
Supplemental figure 8





Supplemental figure 8. Pioglitazone treatment aggravates phenotype of *PTEN* mutants. (**A** and **B**) Representative images of H&E (**A**) or ORO (**B**) stained liver sections of random-fed 8 month old male mice fed with control or Pioglitazone (200mg/kg) containing chow for three months. (**C** and **D**) Relative transcript levels of PPARγ target genes (**C**) and immunoblot analysis of total protein extracts (**D**) from liver tissue of mice treated as in (**A**). Data are means±SEM, n=4. *: $p<0.05$ vs WT and $: $p<0.05$ vs chow food; 1-way ANOVA with Tukey's multiple comparisons test.

Supplemental figure 9



Supplemental figure 9. PPARγ antagonist is therapeutic in *PTEN* mutants. (**A**) Body weight recorded in 6 month old random-fed *PTEN* LKO male mice during treatment with SR2595 (20mg/kg) or placebo (n=3). (**B** and **C**) Relative transcript levels of PPARγ target genes (**B**) and immunoblot analysis of HK2 protein levels (**C**) in livers of 6 month old random-fed *PTEN* LKO male mice treated as in (**A**). Densitometric analysis of Tubulin normalised signal of HK2 is presented as a graph (n=3). Data are means±SEM, *:p<0.05 vs placebo; 2-tailed, unpaired Student's t test. (**D**) Plasmatic free fatty acid levels in mice treated as in (**A**). Data are means ±SEM, n=3. *: p<0.05 vs before treatment, #:p<0.05 vs placebo; 2-tailed, unpaired Student's t test. (**E**) Relative transcript level of PPARα target genes in livers of mice treated as in (**A**). Data are means ±SEM, n=3. (**F**) Hepatocyte size presented as nuclei density scored in a total area of 0.3 mm^2 of liver tissue of mice treated as in (**A**). Data are means±SEM, n=3. *: p<0.05 vs WT and #: p<0.05 vs *PTEN* LKO/placebo; 1-way ANOVA with Tukey's multiple comparisons test. (**G**) Body weight in 12 month old *PTEN* LKO male mice during one month treatment with SR2595 (20mg/kg) or placebo from 11 month of age. Data are means±SEM, n=6. (**H**) Relative liver weight in 12 month old *PTEN* LKO male mice treated as in (**G**). Data are means±SEM, n=6. *:p<0.05 vs WT, #:p<0.05 vs placebo; 1-way ANOVA with Tukey's multiple comparisons test. (**I**) Size distribution of macroscopic lesions (1-5 mm and above 5mm) in livers of mice treated as in (**G**). Data are means±SEM, n=6. *:p<0.05 vs placebo; 2-tailed, unpaired Student's t test. (**J** and **K**) Hepatocyte proliferation scored as a relative number of BrdU+ hepatocytes (**J**) and hepatocyte apoptosis scored as a relative number of TUNEL+ hepatocytes (**K**) in the hepatic lesions in mice treated as in (**G**). Data are means±SEM, n=50 lesions per group. *:p<0.05 vs placebo; 2-tailed, unpaired Student's t test.

Supplemental table 1. List of antibodies

Antigen	Commercial reference, Company
PPARγ	CS 2435, Cell Signaling
HK2	CS 2867, Cell Signaling
PKM2	CS 4053, Cell Signaling
aP2	CS 3544, Cell Signaling
ACC	CS 3662, Cell Signaling
FAS	CS 3180, Cell Signaling
ATPCL	CS 4332, Cell Signaling
HNF1α	CS 12425, Cell Signaling
HNF1α	Sc-6548, Santa Cruz
FABP1	Sc-50380, Santa Cruz
HNF4α	CS 3113, Cell Signaling
Albumin	A0433, Sigma-Aldrich
PTEN	CS 9188, Cell Signaling
Akt pS473	CS 4051, Cell Signaling
Akt2 pS473	CS 8599, Cell Signaling
ATPCL pS454	CS 4331, Cell Signaling
Phospho-Akt Substrate (RXXS*/T*)	CS 9614, Cell Signaling
Pras40	CS 2691, Cell Signaling
Pras40 pT246	CS 2997, Cell Signaling
PCNA	CS 2586, Cell Signaling
Akt2	CS 5239, Cell Signaling
PKL	ab137787, Abcam
Enolase1	Sc-7455, Santa Cruz
GAPDH	Sc-25778, Santa Cruz
Actin	A5441, Sigma-Aldrich
Tubulin	T9026, Sigma-Aldrich
Lamin A/C	CS 2032, Cell Signaling
Ki67	PA5-19462, Thermo
HNF1α pS247	Custom made, Cell Signaling

Supplemental table 2. List of primers

Primers for ChIP	
Hs_Albumin_HRE_S	TGGCAGCCAATGAAATACAA
Hs_Albumin_HRE_AS	TGTGGGGTTGACAGAAGAGA
Hs_PPARg_HRE#1_S	TCAAATTGCTTTGGGTTTGTGCAG
Hs_PPARg_HRE#1_AS	TTAAGAATTATTGCTGATTATTGAAATCTAAAACAC
Hs_PPARg_HRE#2_S	TGTTGGCCAGGCTGGTCTCGAACTCCTGACCTCAGG
Hs_PPARg_HRE#2_AS	ACAAGGCCTGCTCTCATTAACTTCTAC
Hs_PPARg_HRE#3_S	AGCAGTCATTAACAGACTCAATTG
Hs_PPARg_HRE#3_AS	AGGATCCTGAAACAGTGCAGATACA
Hs_PPARg_HRE#5_S	TAGGTATGGGCTACCCTCGTG
Hs_PPARg_HRE#5_AS	TGCCTGGGTATTTTCTTCACTCT
Hs_PPARg_HRE#6_S	TCCTGGACATCATTTACCACTG
Hs_PPARg_HRE#6_AS	AGACCAAACAAGTTCAGATATC
Hs_PPARg_HRE#7_S	TGCTTGAGTCAAAAGGAGAGCC
Hs_PPARg_HRE#7_AS	TGAGGAGCGGGATTTAGCTGT
Hs_PPARg_HRE#9_S	AGTAGATGAAGAGTCCAGAAGTGAG
Hs_PPARg_HRE#9_AS	ACTACAAAGTAATCCAGACACGATGG
Primers for qPCR	
Mm_pinin_S	ACCTGGAAGGGGCAGTCAGTA
Mm_pinin_AS	ATCATCGTCTTCTGGGTCGCT
Mm/Hs_Cyclophilin_S	CAGGTCCTGGCATCTTGTCC
Mm/Hs_Cyclophilin_AS	TTGCTGGTCTTGCCATTCCT
Mm_RnS18_S	AGTCCCTGCCCTTTGTACACA
Mm_RnS18_AS	CGATCCGAGGGCCTCACTA
Mm_PPARg_S	TGTGGGGATAAAGCATCAGGC
Mm_PPARg_AS	CCGGCAGTTAAGATCACACCTAT
Mm_Pparg1mRNA	CAGGAGCCTGTGAGACCAACA G
Mm_Pparg2mRNA	GGTGAAACTCTGGGAGATTCT CC
Mm_PpargmRNA_Common	GTGTGGAGCAGAAATGCTGGA G
Mm_Cidec_S	ATGGACTACGCCATGAAGTCT
Mm_Cidec_AS	CGGTGCTAACACGACAGGG
Mm_CD36_S	TGGCTAAATGAGACTGGGACC
Mm_CD36_AS	ACATCACCACTCCAATCCCAAG
Mm_aP2_S	AAGGTGAAGAGCATCATAACCCT
Mm_aP2_AS	TCACGCCTTTCATAACACATTCC
Mm_Fasn_S	GCTGGCATTCGTGATGGAGTCGT
Mm_Fasn_AS	AGGCCACCAGTGATGATGTAACTCT
Mm_HK2_S	TGATCGCCTGCTTATTCACGG
Mm_HK2_AS	AACCGCCTAGAAATCTCCAGA
Mm_PKM2_S	TCGCATGCAGCACCTGATT
Mm_PKM2_AS	CCTCGAATAGCTGCAAGTGGTA
Mm_HNF1a_S	GCCCTACCTGATGGTTGGAG
Mm_HNF1a_AS	CCCATCGTCATCCGTGTCAT
Mm_FABP1_S	AGTACCAATTGCAGAGCCAGGAGA

Mm_FABP1_AS	GACAATGTCGCCCAATGTCATGGT
Mm_PAH_S	GCGGTTTCCGTGAAGACAAC
Mm_PAH_AS	ACGACAGTAAGCCAGCAACA
Mm_FGB 1_S	ACGAGGCCAGCAAATACCAA
Mm_FGB 1_AS	GTTCTGTTCTCCCCCACCAG
Mm/Hs_HNF4a_S	GGTAGGGGAGAATGCGACTC
Mm/Hs_HNF4a_AS	AAACTCCAGGGTGGTGTAGG
Mm_LCAD_S	TTTCCGGGAGAGTGTAAGGA
Mm_LCAD_AS	ACTTCTCCAGCTTTCTCCCA
Mm_AOX_S	GGGAGTGCTACGGGTTACATG
Mm_AOX_AS	CCGATATCCCCAACAGTGATG
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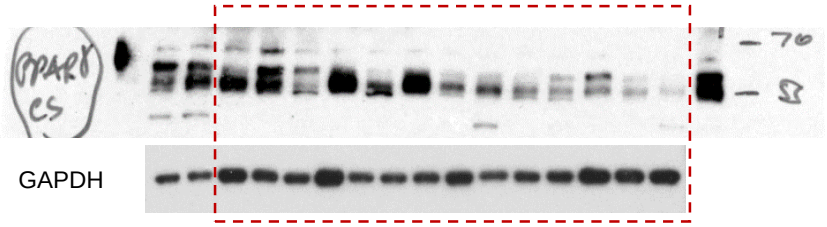
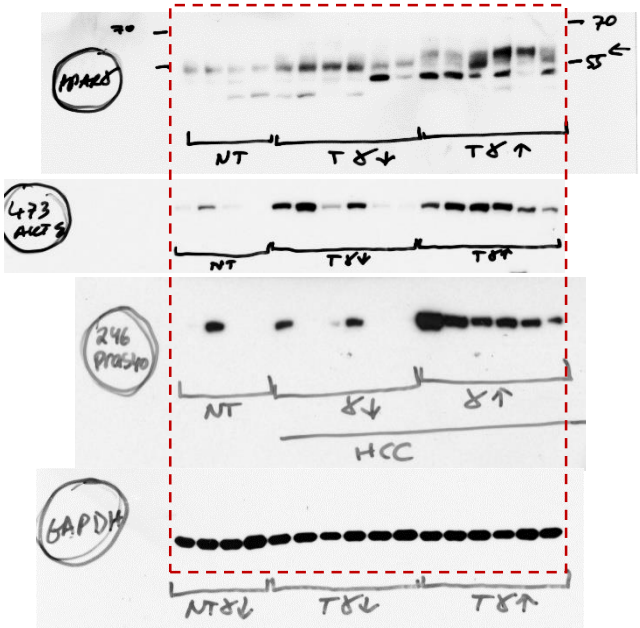
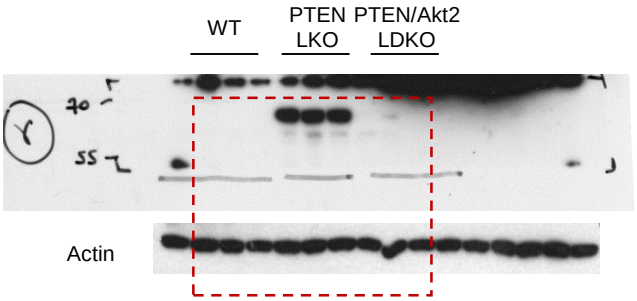
Full unedited gel for Figure 1C



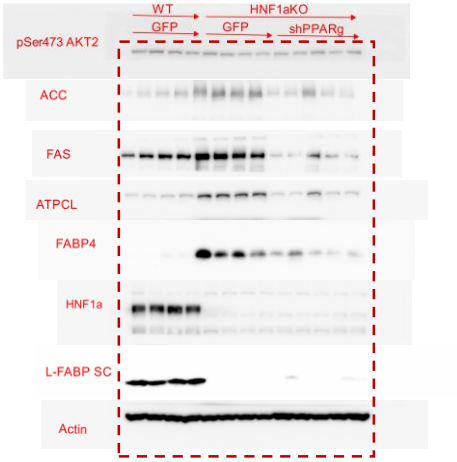
Full unedited gel for Figure 1D



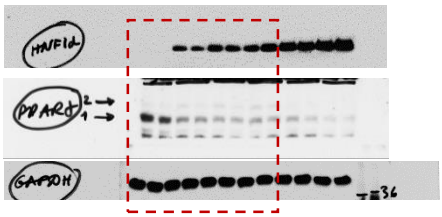
Full unedited gel for Figure 2D



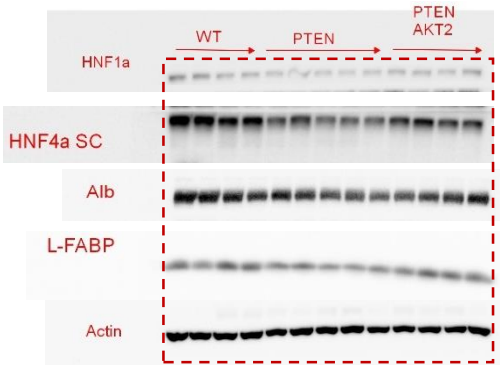
Full unedited gel for Figure 3H



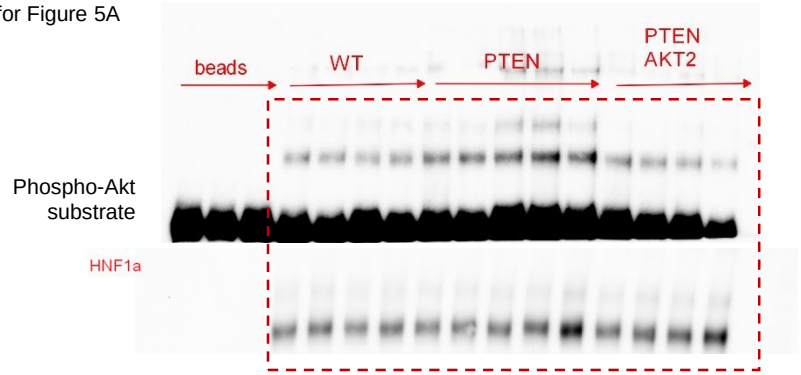
Full unedited gel for Figure 4D



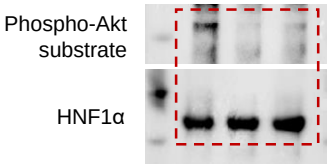
Full unedited gel for Figure 4F



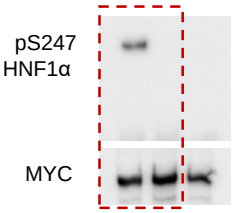
Full unedited gel for Figure 5A



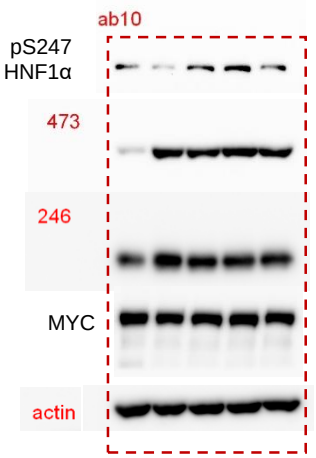
Full unedited gel for Figure 5B



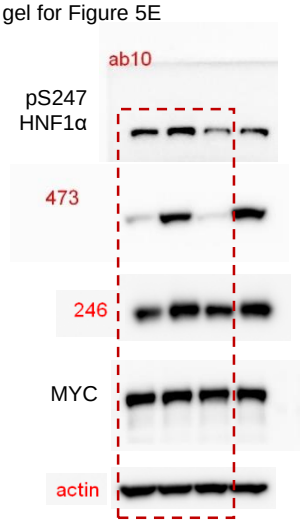
Full unedited gel for Figure 5C



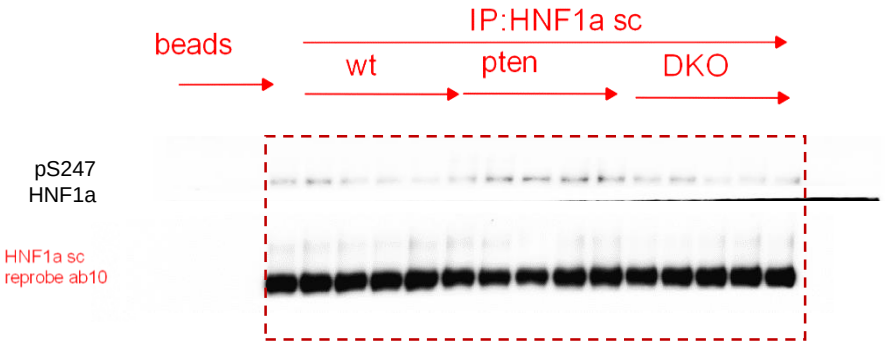
Full unedited gel for Figure 5D



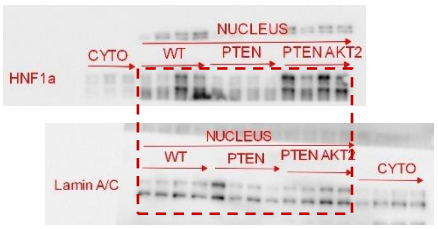
Full unedited gel for Figure 5E



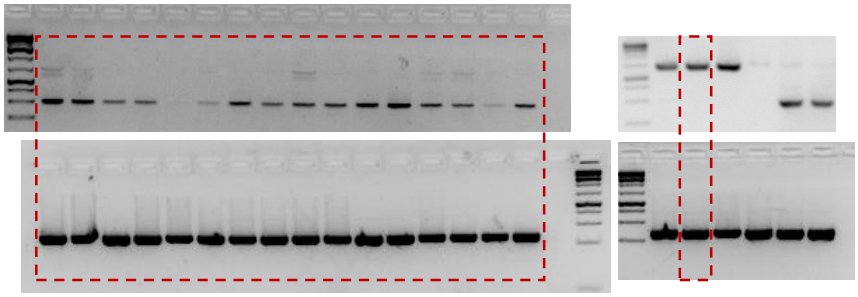
Full unedited gel for Figure 5F



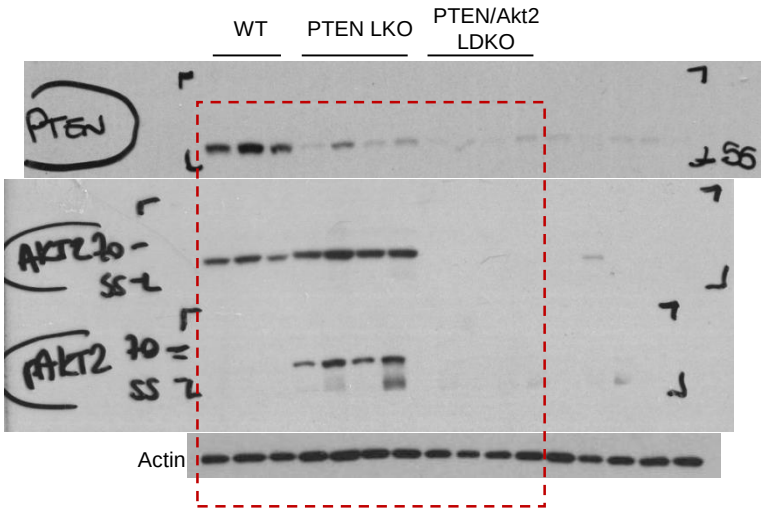
Full unedited gel for Figure 5G



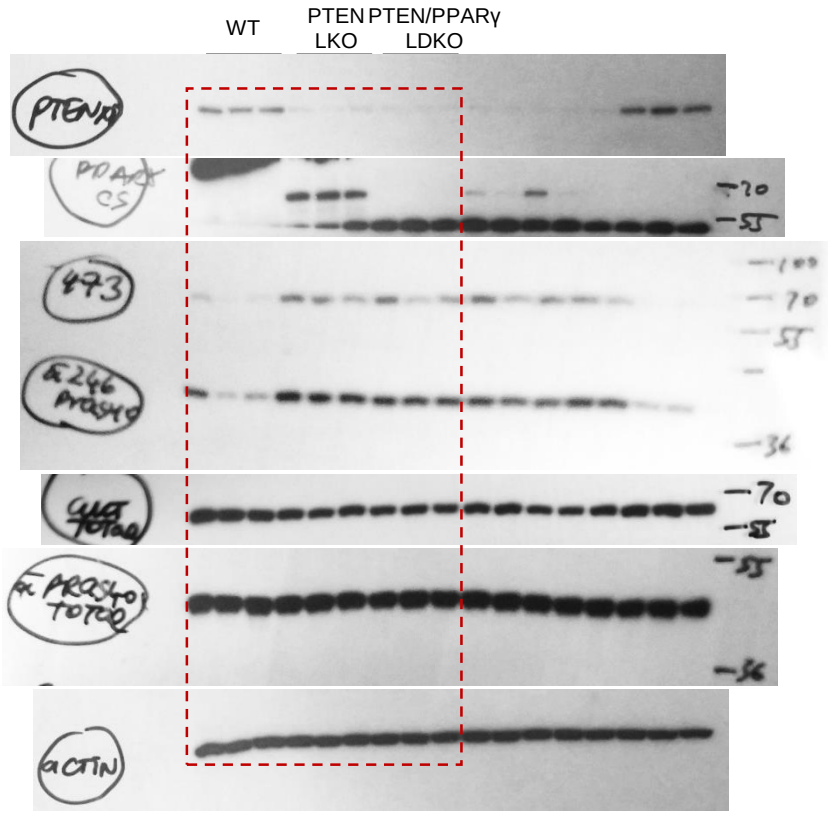
Full unedited gel for Supplemental Figure 2D



Full unedited gel for Supplemental Figure 3A



Full unedited gel for Supplemental Figure 3B



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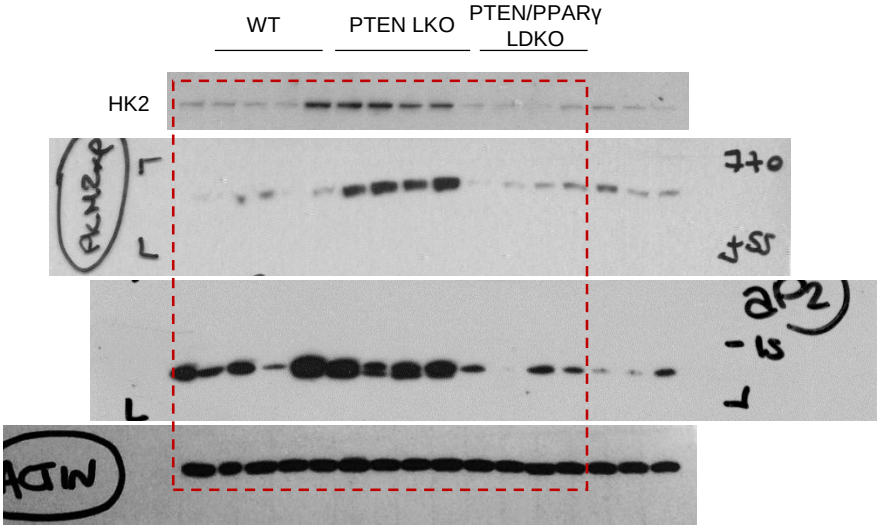

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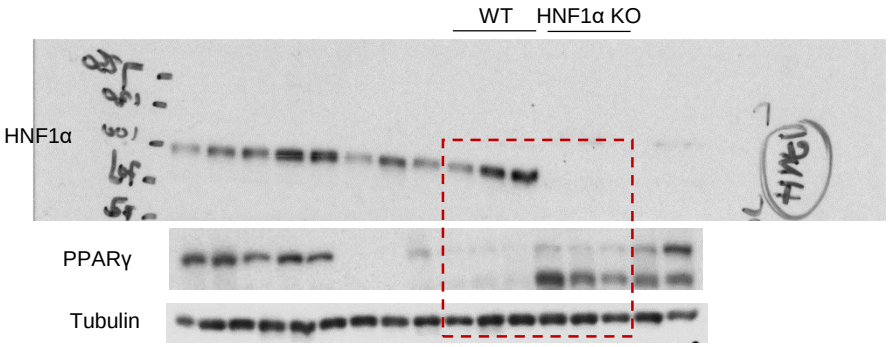

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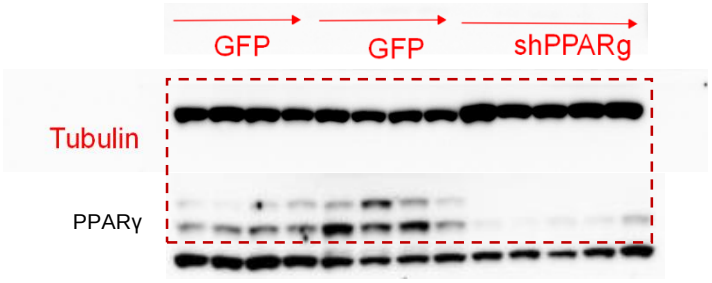
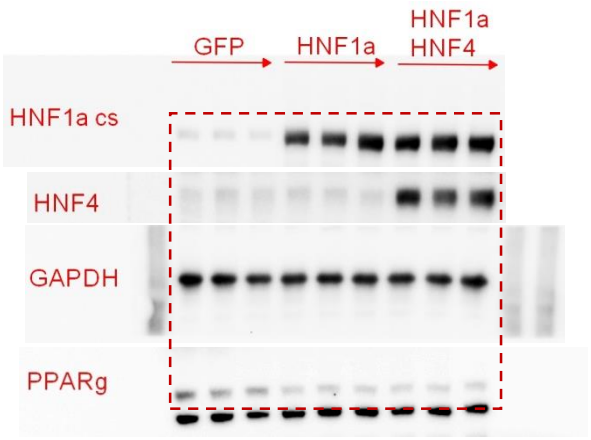


Full unedited gel for Supplemental Figure 6C



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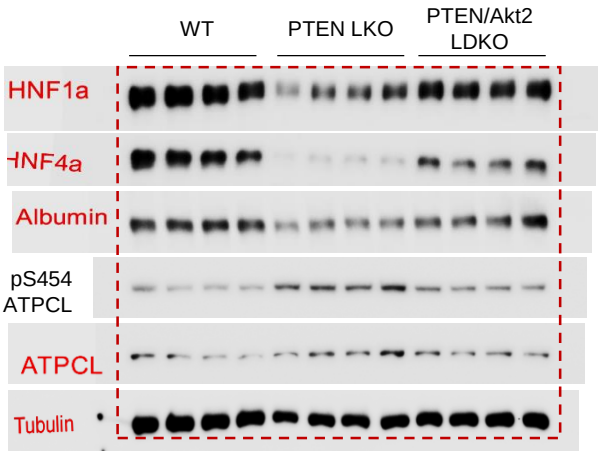
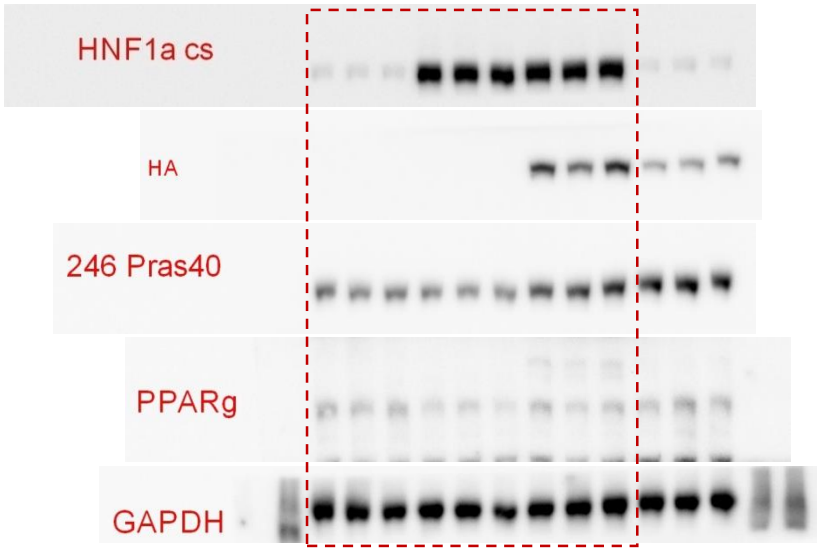
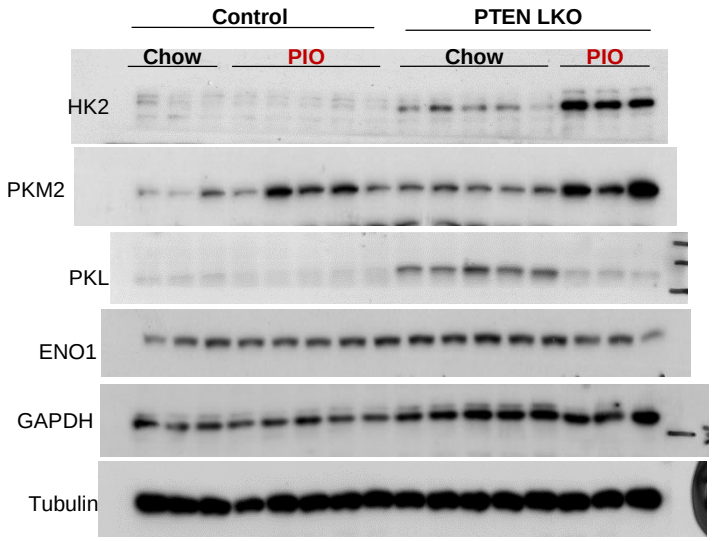


Full unedited gel for Supplemental Figure 6G



Full unedited gel for Supplemental Figure 8D



Full unedited gel for Supplemental Figure 9C